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BIOCHEMICAL TESTS FOR BACTERIAL CHARACTERIZATION. 1. HIPPURATE TEST

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Bacterial characterization is dependent on observations and tests that are both reliable and reproducible. Recently, a series of micromethods using growing cultures was described by Weaver and his colleagues (Arnold and Weaver, 1948; Hannan and Weaver, 1948; Morse and Weaver, 1950; Fabrizio and Weaver, 1951; Bachmann and Weaver, 1951; Hargrove and Weaver, 1951; for review see Cowan, 1953a) and another series using living suspensions by Clarke and Cowan (1952), Cowan (1953b, d) and by Shaw and Clarke (1955). Experience has shown that some of these microtests can be improved by changes in technique (Clarke, 1953; Cowan, 1953c) and that there is a need for new tests to improve bacterial characterization. The present paper concerns one of the more recently developed microtests.

Hajna and Damon (1934) described a medium, with sodium hippurate as the only carbon source, which could be used to distinguish between Aerobacter aerogenes and A. cloacae, either by observing growth or by the persistence of a precipitate produced by the action of ferric chloride with benzoic acid; hippuric acid gives a precipitate which is soluble in excess of ferric salt. In use the test had two disadvantages: (i) like Koser's citrate test the result could be vitiated by carry-over of the medium, (ii) the uninoculated medium sometimes had a slight precipitate which made scanty growth difficult to recognize. Furthermore the ferric salt test depended on a preliminary titration of each batch of medium to find the optimal amount of ferric salt solution.

In a trial of the hippurate medium, changes in pH value of the medium were observed by adding a few drops of indicator solution to duplicate tubes. When the uninoculated medium had a pH value of 6.4, the medium in a positive test had a pH of 6.8-7.2 after 4 days growth, and 7.4-7.6 after 7 days.

From this preliminary experiment was developed a test in which a colour change indicated a positive result; this was easier to read than the precipitate test or the presence or absence of growth.

Media and Methods

A saline suspension containing about 10^8 organisms per ml. was inoculated with a straight wire into tubes containing about 5 ml. of medium prepared according to the formula of Hajna and Damon (1934). Cultures were incubated at 30°C and growth estimated by observing opacity. The hydrolysis of sodium hippurate was detected by adding half-saturated ferric alum solution; the amount to be added was previously determined as the least amount which completely redissolved the precipitate formed when lesser amounts of ferric alum were added to the uninoculated medium.

For the microtest, suspensions were made as described by Clarke and Cowan (1952). Sodium hippurate solutions were sterilized either by Seitz filtration or by autoclaving at 10 lbs for 10 minutes. Sorensen's phosphate buffer at pH 6.8 was used at 0.025 M concentration.

Experimental

Cultures in Hajna and Damon's medium were inoculated in duplicate, incubated, and the opacity of the cultures observed daily. After 7 days incubation ferric alum was added to one set of tubes and bromthymol blue solution to the other. Table 1 gives examples of typical results; strains of Escherichia coli did not grow in the medium and did not produce any change in reaction; on the other hand Klebsiella cultures reacted in different ways, some growing and changing the reaction of the medium, others not growing in it. Neither the opacity of the medium nor the ferric alum precipitate correlated absolutely with the change in pH value, but it seemed likely that the pH change could be used as an indicator of a positive hippurate test, and that by including bromthymol blue in the medium, daily observations could be made. However, before this was tried out an experimental micromethod was developed and the use of growing cultures was abandoned.

Table 1. The use of (a) opacity, (b) ferric alum precipitate and (c) pH value to detect hippurate-positive tests.

Strain No.	Genus and species	Opacity	Ferric alum	pH value
-	Uninoculated medium			6.4
2794	Klebsiella pneumoniae	++++	++	7.6
204	Klebsiella pneumoniae	-	-	6.4
5051	Klebsiella ozaenae	-	-	6.4
5055	Klebsiella pneumoniae	++	++	6.8
8819	Escherichia coli	-	-	6.4
9194	Klebsiella serotype	+++	++++	7.2

+ to ++++ = increasing degrees of opacity or bulk of precipitate

For the microtest the optimal concentration of sodium hippurate was found to be 1 per cent. Table 2 shows the pH values produced by suspensions of living organisms in different concentrations of hippurate; the suspension of the positive culture (A) had a pH of 6.4 but in 1 per cent hippurate it produced a value of 7.4. In stronger concentrations of hippurate the reaction became more acid, and in 5 and 10 per cent hippurate a value of 6.0 showed that the products of the suspension-substrate reaction were more acid than the suspension. With concentrations less than 1 per cent, positive cultures produced only a small difference between the

Table 2. Effect of varying the concentration of sodium hippurate in the microtest.

(Hippurate solution, 0.06 ml.; buffer solution, 0.06 ml.; indicator solution, 0.04 ml.; suspension, 0.02 ml.)

Hippurate Concentration	pH values after 24 hours at 37°C. Culture A	Culture B
10 per cent	6.0	6.2
5 per cent	6.0	6.2
2.5 per cent	6.4	6.2
1 per cent	7.4	6.4
none	6.4	6.6

Culture A gave a positive reaction in the Hajna and Damon medium; culture B was negative.

test and the control tubes. Suspension of the negative culture (B) had an initial pH value of 6.6 and lower values after incubation with hippurate for 24 hours at 37°C. With 1 per cent sodium hippurate the optimal starting pH value for the test was found to be between 6.4 and 6.8. Unlike most of the other microtests, the results were easier to read when the density of the suspension was rather less than the standard (10^9 organisms per ml.), possibly because the colour of the indicator was masked by the opacity of the suspension.

The microtest finally developed and used in a routine trial was as follows: 1 per cent sodium hippurate, 0.06 ml.; 0.025 M-phosphate buffer, pH 6.8, 0.06 ml.; bromthymol blue indicator solution (British Drug Houses Ltd., London), 0.04 ml.; suspension 0.02 ml. Each test was controlled by a tube containing 0.06 ml. distilled water in place of hippurate solution. Exact volumes were not critical and the test

Table 3. Examples of positive and negative microtests.

Organism	Test	Control
	Na hippurate 0.06 ml.	none
	Water none	0.06 ml.
	Buffer 0.06 ml.	0.06 ml.
	Indicator 0.04 ml.	0.04 ml.
	Suspension 0.02 ml.	0.02 ml.
	pH value after 24 hours at 37°C.	
Escherichia coli (9001)	6.8	7.0
Klebsiella aerogenes (243)	7.4	7.0
Klebsiella pneumoniae (5055)	7.4	7.0
Klebsiella pneumoniae (7242)	7.2	6.8
Klebsiella ozaenae (5050)	6.8	6.8
Cloaca cloacae (5920)	6.8	6.8

could be made with uncalibrated dropping pipettes. Tubes were incubated at 37°C for 24 hours; the pH value of both the test and the control tubes was then estimated by comparison with standard capillary tubes (B.D.H. Capillator). In a negative test there might be no difference in pH value between test and control tubes or the test might be more acid than the control; in a positive result the test would be more alkaline than the control. An arbitrary value of + 0.4 pH unit for the difference between the test and the control was taken as the minimum for a positive test. Examples are shown in Table 3.

Table 4. Frequency of positive and negative hippurate microtests.

	+	-
Escherichia coli	0	14
Escherichia freundii	0	4
Klebsiella aerogenes	5	1
Klebsiella pneumoniae	8	0
Klebsiella ozaenae	2	4
Klebsiella rhinoscleromatis	1	3
Klebsiella serotype	7	0
Cloaca cloacae	3	10
Proteus sp.	0	3

The microtest was carried out in routine tests and the results (Table 4) showed that the test might have some value in the classification of members of the family *Enterobacteriaceae*. All strains tested of *Escherichia coli* and *E. freundii* were hippurate-negative, as were three strains of *Proteus* and one each of *Salmonella* and *Shigella*. Trials with these genera were not pursued as the test was primarily intended to help in distinguishing *Aerobacter aerogenes* from *A. cloacae* and from *Klebsiella pneumoniae* (these names being used without prejudice). Apart from motility and gelatin-liquefaction, the distinguishing features between *A. aerogenes* and *A. cloacae* are not well established, and Edmunds (1954) classifies the only hippurate-negative strain of *A. aerogenes* (NCTC 8348) as *A. cloacae*. I find it difficult, and generally impossible, to distinguish between *Aerobacter aerogenes* and *Klebsiella pneumoniae*, and in the tables I have included organisms sent to this Collection as *Bacterium aerogenes* or *Aerobacter aerogenes* in the genus *Klebsiella*. It was hoped that the hippurate test might help to distinguish *A. aerogenes* from *K. pneumoniae*, but this it fails to do.

Discussion

The hippurate microtest is easier to read than the corresponding test using cultures growing in Hajna and Damon's medium. In the two tests the criteria for positivity are different and it is even possible that the end-products tested for are different; thus the tests may not be strictly comparable. Be that as it may, when using the microtest most strains labelled Aerobacter cloacae were negative, whereas those labelled A. aerogenes or Klebsiella pneumoniae were positive. Our strains of A. cloacae form a heterogeneous group so that it was not surprising that the hippurate test was not uniformly negative. At the present time each worker has his own criteria for making the diagnosis of Aerobacter (or Cloaca) cloacae, and the hippurate test may yet find a place in making the distinction between this species and A. aerogenes. In the other problem of distinguishing A. aerogenes from K. pneumoniae, the evidence obtained in the limited trial reported here does not suggest that the test will be of much value.

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CHANGE IN THE METHODS OF DESIGNATION OF NEW SALMONELLA SEROTYPES

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At the September 8, 1953 meeting of the Enterobacteriaceae Subcommittee held in Rome, Italy, during the VI Congress for Microbiology, a majority of those present voted in favor of the following Addendum to the Subcommittee Report:

"It is the recommendation of the Enterobacteriaceae Subcommittee that from the date of publication of this report all new serological types of Salmonella should be described by formula only and not by name."

This Addendum was duly recorded in the Minutes of the September 8, 1953 Subcommittee meeting and was published in the Enterobacteriaceae Subcommittee Reports (Internatl. Bull. Bact. Nomen. and Tax. 4:73. 1954). Some months after publication of the Subcommittee Reports, several Subcommittee members requested the Subcommittee Chairman to ask the membership to consider cancellation of the above-mentioned Addendum. Therefore, at the Chairman's request, the Secretary sent (December 21, 1954) a ballot to each Subcommittee member and asked for a vote either in favor of or against cancellation of the decision made on September 8, 1953 to describe new Salmonella serotypes by formula only. It was pointed out at that time that the vote was for or against an emendation of the Minutes of the September 8, 1953 Subcommittee meeting and a consequent change in the Report. On February 1, 1955, the Secretary informed the Chairman and the Subcommittee membership of the result of the voting, which was as follows:

13 voted in favor of cancellation (The Subcommittee Chairman also favors cancellation, but since there was a majority opinion, the Chairman's vote was not required).

2 voted not in favor of cancellation. 1 abstained.

The results of the voting indicated clearly that the majority of the Subcommittee membership favored cancellation of the above-mentioned Addendum to the Minutes of the September 8, 1953 Subcommittee meeting in Rome, deletion of the Addendum from the Subcommittee Report (lines 5-8, page

73), and a return to the earlier method of describing new Salmonella serotypes by a name and their antigenic formula.

The attached list of names and references for Salmonella serotypes that were described by formula only was supplied by Dr. F. Kauffmann.

ADDENDUM

Names of Salmonella Serotypes Published Without Names

Nomina nova proposed by Dr. F. Kauffmann

Salmonella brazzaville = 6,7:b:1,2
Typed by LeMinor, Merveille, Bascoulergue and Audebaud (Annal. Inst. Pasteur 87:105. 1954).

Salmonella kapemba = 9,12:1,v:1,7
Typed by Kauffmann, Delville, Bouckaert, and Ballion (Acta Path. et Microbiol. Scand. 35:307. 1954).

Salmonella minneapolis = (3),(15),34:e,h:1,6
Typed by Edwards, Kauffmann, and McWhorter (Acta Path. et Microbiol. Scand. 35:67. 1954).

Salmonella boecker = 6,14:1,v:1,7
Typed by Boecker and Kauffmann (Acta Path. et Microbiol. Scand. 34:101. 1954).

Salmonella landau = 30:i:1,2
Typed by Günther and Hanser (Zbl. Bakt. I Orig. 161:363. 1954).

Salmonella kingabwa = 43:y:1,5
Typed by Kauffmann and Vendepitte (Acta Path. et Microbiol. Scand. 35:71. 1954).

Salmonella hull = 16:b:1,2
Typed by Alexander, Douglas, and Taylor (Monthly Bull. Min. Health, London. 13:117. 1954).

Salmonella manila = 3,15:z_{10}:1,5
Typed by Edwards, Kauffmann, and McWhorter (Acta Path. et Microbiol. Scand. 35:67, 1954).

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RELAPSING FEVER SPIROCHETES: THE PRESENT STATUS OF BORRELIA VENEZUELENSIS BRUMPT AND BORRELIA NEOTROPICALIS BATES AND ST. JOHN

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Following a long series of experiments with ticks and spirochetes of Panama, Bates, Dunn, and St. John reported (1921) that the human tick, Ornithodoros talaje, had been demonstrated to be the transmitting agent of relapsing fever by human experimentation. The causal organism was not named in this paper. Following further studies on the spirochete and comparison with several other species, Bates and St. John (1922) proposed the name Spirochaeta neotropicalis for the relapsing fever spirochete of the neotropical region. Meanwhile Brumpt (1921) had given the name Treponema venezuelense to the spirochete transmitted by Ornithodoros rudis.

In an attempt to clarify the confusion which exists in the use of the two species names, Borrelia venezuelensis and Borrelia neotropicalis, currently applied to the spirochete(s) of neotropical relapsing fever, many of the extensive reports from Panama have recently been reviewed. There were two problems: (1) to determine whether B. neotropicalis was actually applied to the spirochete normally transmitted by O. talaje, as originally reported, or to the spirochete normally transmitted by O. rudis, and, (2) to determine the priority of publication of the two specific epithets if they were both applied to the spirochete normally transmitted by the same species of Ornithodoros.

Identity of the ticks concerned.

Ornithodoros talaje was described from Guatemala by Guérin-Méneville in 1849 and Ornithodoros rudis from "Nova Granada"[2] by Karsch in 1880. Neither description contained

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[2]'Nova Granada' is the geographic name that was once applied to the general region of Colombia and Panama.

what are not considered as sufficient characters to delimit a species. In 1921, Brumpt described the vector from Venezuela as Ornithodoros venezuelensis, this is now generally accepted as a synonym of O. rudis Karsch. It was not until 1927 that Dunn reported that an error had been made in the identification of the ticks in Panama, and that the tick formerly reported as O. talaje was O. rudis. It thus becomes clear that the epithet neotropicalis was not given to the spirochete transmitted by O. talaje, but rather to the spirochete transmitted by O. rudis. In other words, Borrelia venezuelensis and B. neotropicalis are synonyms.

Priority of the species names.

Priority as between Borrelia venezuelensis and B. neotropicalis was difficult to establish. In a letter from the late Prof. Brumpt, dated 1 July 1950 from Paris, he stated:

> En ce qui concerne le spirochète vénézuelien, c'est en 1921, dans le nouveau traité de Médecine, que je lui ai donné le nom Treponema venezuelense, le genre Treponema ayant été accepté par divers auteurs comme devant englober les différentes spirochètes des fièvres récurrentes. Il est probable que le date de 1922 a été donné au moment de la publication du traité de Médecine, mais j'ai créé mon espèce en 1921.

However, according to the rules governing priority of names, a name must be validly published and available; its date is fixed by the date of publication. The writer's edition of Nouveau Traité de Medecine, labelled in pencil as the 1922 edition, and in which Treponema venezuelense Brumpt 1921 is listed, proved to be the 1925 edition after checking the paginations of the several editions. Numerous attempts were made to obtain the 1921 edition from University libraries and from the Surgeon-General's Library, without success.

During these searches we found a reference to a thesis by Lavier (1920-21). The thesis was submitted to the Faculty of Medicine of Paris for the Doctorate in Medicine. A copy was readily obtained from the Surgeon General's Library. The species name Treponema venezuelense Brumpt 1921 is mentioned with a reference to Les Spirochetóses, Brumpt, Nouveau Traité de Médecine, fascic. 4, Paris, Masson, 1921. The priority of the specific epithet in Borrelia venezuelensis

over that in B. neotropicalis would seem to be established, and the latter name is a synonym of the former.

Following is a revision of the ascriptions and synonymy.

Borrelia venezuelensis Brumpt 1925.
Treponema venezuelense Brumpt 1921.
Spirochaeta neotropicalis Bates and St. John 1922.
(Spirochete of Panama) St. John and Bates 1922.
Borrelia neotropicalis Bates and St. John 1922.

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THE DESIGNATION OF CORYNEBACTERIUM UREAFACIENS KREBS AND EGGLESTON AS ARTHROBACTER UREAFACIENS (KREBS AND EGGLESTON) COMB. NOV.

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In 1937 Dubos and Miller (1) isolated a small Gram-positive bacterium which grew readily on all common laboratory media and which was of particular interest to them in that when grown on creatine or creatinine as a sole source of carbon and nitrogen, urea accumulated in the substrate. At that time they designated their culture simply as Culture NC. In 1939, Krebs and Eggleston (2) confirmed the biochemical observations of Dubos and Miller; they also extended the description of the bacterium and named it Corynebacterium ureafaciens. Later, Dubos and Miller sent their initial isolate to the American Type Culture Collection, under the name Corynebacterium creatinovorans. This name appeared in the following ATCC catalogue of cultures (3) and it has also appeared in some more recent literature (4, 5).

Inasmuch as Krebs and Eggleston employed the culture of Dubos and Miller, the two differently named cultures obviously are identical. C. ureafaciens has been of interest primarily to workers engaged in biochemical studies. Its relationship to other species of Corynebacterium, and the question of whether it should be placed in the genus Corynebacterium or in one of the closely related genera now recognized for the soil corynebacteria (6), have not heretofore been considered. It is my purpose here to call attention to the close similarity of the soil isolate Corynebacterium ureafaciens to certain soil corynebacteria now recognized in the genus Arthrobacter, and to propose that the species be transferred to Arthrobacter.

Observations have been made on C. ureafaciens no. 7562 received from the American Type Culture Collection. This culture meets the description given by Krebs and Eggleston, particularly in its irregular cell morphology, uneven staining, nonmotility, yellow pigmentation, ability to grow on inorganic media, gelatin liquefaction, failure to reduce nitrates, catalase production, and failure to produce urease.

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In the course of comparative screening of a number of corynebacteria of soil, plant, or animal origin, it was quickly found that the great majority of them were culturally dissimilar from Corynebacterium ureafaciens. These cultures are accordingly dismissed from any individual consideration in order that attention can be focused on a few cultures that have shown less marked cultural dissimilarity. These cultures are three strains of Arthrobacter aurescens isolated by Conn and Dimmick (7), and an unnamed soil corynebacterium designated T178, isolated by Topping (8) in Scotland. Conn and Dimmick, although they did not publish a description of A. aurescens, did refer (p. 350) to a yellow chromogenic bacterium which occurred in soil and which they stated undoubtedly belonged in the same genus as A. globiforme. Cultures sent by Conn to the writer in 1950 were labeled A. aurescens. These cultures were studied by Clark (9) and later by Phillips (10).

These cultures, as well as Corynebacterium ureafaciens, are capable of growing on creatine, creatinine, and uric acid as a sole source of carbon and nitrogen, and they are urease negative. Their growth responses on common laboratory test media are in agreement with those of C. ureafaciens. All produce a yellow pigmentation, which varies somewhat in intensity.

The similarity of these cultures to C. ureafaciens points to the desirability of placing them all in the same genus, and in a single species. The writer has discussed previously (6) the desirability of recognizing the genus Arthrobacter Conn to accommodate the numerous corynebacteria of soil origin that as a rule grow readily on inorganic media and whose inclusion in Corynebacterium does not seem desirable, particularly since they differ quite markedly from the type species of that genus. Accordingly, the designation of Corynebacterium ureafaciens Krebs and Eggleston as Arthrobacter ureafaciens comb. nov. is herewith proposed.

Arthrobacter ureafaciens (Krebs and Eggleston) comb. nov.
Corynebacterium ureafaciens Krebs and Eggleston 1939.
Corynebacterium creatinovorans Dubos and Miller 1949? (not validly published).
Arthrobacter aurescens Conn and Dimmick (?) 1948.

SUMMARY

Arthrobacter ureafaciens comb. nov. is proposed for certain yellow-pigmented soil corynebacteria that grow readily on creatine and uric acid media but fail to produce urease. These bacteria have previously been recognized under the name *Corynebacterium ureafaciens*, *C. creatinovorans*, *Arthrobacter globiforme* var. *aurescens*, and *A. aurescens*.

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BREVIBACTERIUM HELVOLUM (ZIMMERMANN) COMB. NOV.

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In 1890, Zimmermann (7) described an organism isolated from Chemnitz tap water to which he gave the name Bacillus helvolus. The organism was reported to be a nonsporulating, Gram-positive rod, occurring usually in pairs, as well as in fours or longer chains. The cells were about 0.5 microns in width and usually 1.5 to 2.5 microns in length though at times they reached a length of 4.5 microns. The rods were stated to rotate on their long axis. On agar, gelatin and potato the organism grew well, with cultures of a bright yellow color, gelatin being slowly liquefied, while broth became turbid with a yellowish-white sediment. The organism grew best at room temperature.

In view of the inability of the species to produce spores, Lehmann and Neumann (6) proposed a new combination, naming the organism Bacterium helvolum. These authors also added certain characters to the original description from a study of a culture isolated from air that agreed with Zimmermann's culture. Lehmann and Neumann furthermore reported their strain to be able to coagulate milk and produce hydrogen sulfide, but incapable of producing indole or of forming gas from glucose. Though confirming Zimmermann's description in other respects, they stated that their culture was nonmotile.

In 1918, the name Corynebacterium helvolum was first used as a new combination for Zimmermann's organism by Kisskalt and Berend (5). The paper in question, which has been widely quoted, provides no comparative experimental data, but states briefly that the authors have recognized Bacterium helvolum, among others, as a diphtheroid.

A further combination for Zimmermann's organism appeared in the first edition of Bergey's Manual of Determinative Bacteriology, 1923, in which the binomial, Flavobacterium helvolum, was first used. The description of the species, which was carried through unaltered to the fifth edition of the Manual, 1939, indicated the organism to be Gram-negative, obviously erroneously, since Zimmermann (7) as well as Lehmann and Neumann (6) had stated it to be Gram-positive.

In a study of saprophytic mycobacteria and corynebacteria isolated from soil, Jensen (4) described a species-group which he designated as Corynebacterium helvolum (Zimmermann) Kisskalt and Berend, with the following names considered as synonyms: Bacillus helvolus Zimmermann, Bacterium helvolum (Zimmermann) Lehmann and Neumann, and Flavobacterium helvolum (Zimmermann) Bergey et al. Jensen's species, which represented the most common soil corynebacterium found, showed the wide range of morphological variations characteristic of the "soil diphtheroids" and though various strains showed certain physiological and cultural differences, such as a color range from cream through greyish yellow to chrome-yellow, Jensen regarded them to be most appropriately grouped as one species. It is not clear why Jensen considered his soil type to be identical with Zimmermann's organism, since he does not appear to have had the latter available for comparison, nor is there anything in the descriptions provided by Zimmermann, Lehmann and Neumann, or Bergey, et al. to indicate the pleomorphism noted in Jensen's species.

In 1928, Conn (2) described, under the name Bacterium globiforme, an organism occurring abundantly in soil which showed pronounced pleomorphism in that it changed from a distinct rod in young cultures to a coccus form on further incubation. The characteristics of the species indicated by Conn suggest a relationship of Bacterium globiforme to the soil corynebacteria later described by Jensen (4). Conn's organism, however, does not appear to have been used for comparison nor to have been considered in relation to its possible identity with any of the species of Corynebacterium named by Jensen.

More recently Conn and Dimmick (3) provided further characterization of Bacterium globiforme which strengthened the belief that their organism was closely related to Jensen's soil corynebacteria and in particular to his Corynebacterium helvolum which was included in the studies reported. The group of organisms typified by Bacterium globiforme was admitted by Conn and Dimmick to be closely related to the genus Corynebacterium. However, these authors considered such soil types to have characteristics sufficiently different from those of the type species as to warrant generic distinction and proposed for their inclusion a new genus, Arthrobacter, with Arthrobacter globiforme as the type species.

Apparently solely on the basis of its chromogenesis, Jensen's Corynebacterium helvolum was regarded as a species distinct from A. globiforme.

In a recent comparative study by the writer of organisms of the "soil diphtheroid" type which might be considered for inclusion in the genus Arthrobacter, proposed for the seventh edition of Bergey's Manual, a culture of Conn's Arthrobacter globiforme as well as one of Jensen's Corynebacterium helvolum were included*. Though Jensen's strain was yellow when received, it had later become nonchromogenic, showing a cream colored growth on agar indistinguishable from that of A. globiforme. Comparative tests, morphological and physiological, showed the cultures to resemble each other so closely that it was impossible to designate any distinguishing feature in which they differed. The findings therefore point to the identity of Jensen's C. helvolum with A. globiforme (Conn) Conn and Dimmick. Though Conn and Dimmick (3) were inclined to consider the yellow pigmentation of Jensen's organism sufficient for species separation, Jensen (4) included in the species strains with a range of pigmentation from cream to yellow, and this fact, considered in conjunction with the loss of color in the strain examined by the writer, leads us to regard the chromogenic strains as variants of A. globiforme. The reason for Jensen's assumption of the identity of this species with Zimmermann's organism is not clear, and doubt of this identity was expressed by Conn and Dimmick.

If the identity of Jensen's organism with A. globiforme is admitted, it is clear that the name C. helvolum as employed by him cannot be regarded as synonymous with that used to designate Zimmermann's species. The morphology of the latter organism, both as described by Zimmermann and also by Lehmann and Neumann, is that of a simple rod without any suggestion of snapping division, branching, or the pleomorphism of the "soil diphtheroids". The earlier descriptions thus differ from that given in the 6th edition of Bergey's Manual in which the organism was removed from Flavobacterium and placed in the genus Corynebacterium, with the description radically altered to conform with that given by Jensen. Though it is to be assumed that Kisskalt and Berend (5)

*The cultures were kindly supplied by Dr. Conn, in 1941 and 1948 respectively.

based their selection of the name Corynebacterium helvolum on the study of a culture of Zimmermann's species there is little to indicate the reason for their change in generic name.

Since Zimmermann's organism is the one for which the specific epithet helvolum is valid, it is reasonable that the description should follow Zimmermann's or that of Lehmann and Neumann who were confident that they were working with the same organism. Though it is uncertain whether Zimmermann's culture is still available the descriptions referred to provide a basis for classification and reidentification.

In a paper read before the VI International Congress for Microbiology, Breed (1) established a new genus, Brevibacterium Breed, to include short, unbranched, Gram-positive, nonspore-forming, rod-shaped bacteria, reproducing by simple cell division, with B. linens (Weigmann) Breed as type species. This new genus will be recognized in the seventh edition of Bergey's Manual, and, with the genus Kurthia Trevisan, which includes larger, peritrichous, Gram-positive rods that grow into filaments, will constitute the new family Brevibacteriaceae. The matter of the most appropriate allocation of Zimmermann's organism was discussed with Dr. Breed, Chairman, Bergey's Manual, who agreed that it is properly placed in the genus Brevibacterium. The bibliographical record of this species is as follows:

Brevibacterium helvolum (Zimmermann, 1890) comb. nov.
Bacillus helvolus Zimmerman, 1890,
Bacterium helvolum Lehmann and Neumann, 1896,
Corynebacterium helvolum Kisskalt and Berend, 1918,
not Corynebacterium helvolum Jensen, 1934,
Flavobacterium helvolum Bergey et al., 1923.

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STATUS OF THE GENERIC NAMES CHLAMYDOZOON VON PROWAZEK AND BORRELINA PAILLOT

A Preliminary Statement

Editorial Board

Several queries have been received relating to the status of the generic names Chlamydozoon von Prowazek and Borrelina Paillot. Opinions to be formulated by the Judicial Commission, in an effort to eliminate present confusion in their use, have been requested. This preliminary statement has been prepared to stimulate discussion and to elicit additional ideas and information. Comments and suggestions will be welcomed, and published if desired.

The generic name Chlamydozoon

The ascription of Chlamydozoon to Halberstaedter and von Prowazek 1907 (see Bergey's Manual 6th Ed. p. 1114) is an error. These authors reported the study of intracellular inclusions in several diseases, including trachoma, yellow disease of the silkworm, carp pox, rabies, vaccinia, scarlet fever, and fowl plague. They point out that the etiologic agents found microscopically in these diseases have nothing in common with the bacteria, and that they are equally to be differentiated from the protozoa. They proposed Chlamydozoa as a name coordinate with Bacteria and Protozoa to designate a taxon to include the organisms under study. They did not, in this paper, name or identify any genus or species by name, though they gave a detailed description of the several organisms evidently considered to be distinct species. The generic name Chlamydozoon should not be ascribed to Halberstaedter and von Prowazek 1907.

Later in 1907, von Prowazek published two papers on the Chlamydozoa. In the first, (1907a) were described what he regarded as the causal organisms of variola, vaccinia, scarlet fever, rabies, fowl plague, trachoma, molluscum contagiosum, bird epithelioma, carp pox, lip disease of the barbel, the yellow disease (jaundice) of the silkworm, and as the possible causes of dog distemper and of foot and mouth disease. For none of these organisms does he propose a species name. He does make the statement:

> "nannte ich diese Mikroorganismen provisorisch Chlamydozoa und stellte sie zwischen Bakterien und Protozoen, ohne vorläufig Genaures über ihre systematische Stellung auszusagen."

His second paper (1907b) included a detailed description of the yellow disease (jaundice) of the silkworm. To the causal organism he gave the name Chlamydozoon bombycis in what may be regarded as a combined genus-species description. His statement is:

> "Ich nenne sie vorläufig Chlamydozoon bombycis."

No other species was named. It seems clear that Chlamydozoon bombycis von Prowazek 1907 is the type species (by monotypy) of the genus Chlamydozoon.

The generic name Chlamydozoon Halberstaedter and von Prowazek was accepted by Rake (1948, p. 1114) and the genus included as the first in the family Chlamydozoaceae Moshkovsky 1945; it was the third family of the order Rickettsiales ascribed to Gieszykiewicz 1939. Three genera were recognized, Chlamydozoon, Miyagawanella, and Colesiota. The type species of Chlamydozoon is designated as Chlamydozoon trachomatis Foley and Parrot (1937b). This ascription is apparently in error. Foley and Parrot did not propose the name but erroneously included it as a synonym. The statement made by them is:

> "nous proposerons en conséquence de designer cette nouvelle espèce de Rickettsia par l'appelation de Rickettsia trachomatis (Halberstaedter et von Prowazek 1907)".

In parenthesis they quote as a synonym:

> "(= Chlamydozoon trachomatis H. et v.P.)."

This citation of a presumed synonym does not constitute valid publication. Rule 12 of the International Bacteriological Code of Nomenclature definitely states:

> "A name of a taxonomic group is not validly published when it is merely cited as a synonym."

It would seem that the Bergey citation should have been Chlamydozoon trachomatis (Foley and Parrot) comb. nov., as the name had not been previously validly published. The name Rickettsia trachomatis Foley and Parrot was first published in the Comptes Rendus Soc. Biologie, Paris (1937a) and not in the Arch. Inst. Pasteur d'Algerie (1937b) as cited in Bergey's Manual (6th Ed. 1948, p. 1114). However, Busacca (1935) had earlier proposed as a name for this organism Rickettsia trachomae. The name was given only in the title of the paper. This name evidently has priority over R. trachomatis. Should the specific epithet trachomae be recognized? It seems to have been validly published. However, the word is misspelled, the genitive form of trachoma is trachomatis, not trachomae. Rule 27 of the International Bacteriological Code of Nomenclature states:

> "The original spelling of a name or epithet must be retained, except in the case of a typographical error, or of a clearly unintentional orthographic error."

It seems reasonably clear that the author regarded the word trachoma as a first declension Latin noun with the genitive trachomae instead of a Greek neuter noun ending in -ma and with the genetive form trachomatis. It would seem appropriate to regard the specific epithet trachomae as an unintentional orthographic error for trachomatis. This would make legitimate the ascription of the specific epithet trachomatis to Busacca, and the logical citation in Bergey's Manual (1948, p. 1114) would have been Chlamydozoon trachomatis (Busacca) comb. nov.

The generic name Chlamydozoon Prowazek has also been used in protozoology, as witness its inclusion in Nomenclator animalium generum et subgenerum (Schulze et al. 1929, p. 664) where the genus is placed in "Protoz. Chlamyd." It should be recalled that the author of the name stated definitely that the chlamydozoa were not protozoa (or bacteria) and that the genus Chlamydozoon should not be regarded as belonging to the protozoa. The question has been raised, "Does the transfer of the genus Chlamydozoon from the Protozoa to the Rickettsiales or to the Virales have any bearing upon the correctness of its use?" Even though the taxon Chlamydozoon had been assigned to the protozoa by an author, transfer by later authors to the bacteria would be legi-

timate. Both botanical and zoological codes make provision for such transfers. But as noted above the author definitely declined to place it in the protozoa.

Inasmuch as von Prowazek included only one named species, Chlamydozoon bombycis, in his genus, this is the name of the type species. True, he described several other organisms including that causing trachoma, but he named no others. The species C. bombycis is not included in the revision of the genus Chlamydozoon in Bergey's Manual (1948, p. 1114). A genus cannot, under the rules, be redefined to exclude the type species. It would seem that the generic name Chlamydozoon as used in Bergey's Manual is incorrect, and, if the indicated delimitation is recognized, the genus should be renamed. Obviously, under the rules, the family name Chlamydozaceae, as here used, is also incorrect, and if the taxon is to be recognized, should be replaced by another.

It was noted above that von Prowazek in giving the names Chlamydozoa and Chlamydozoon characterized them as "provisorisch" or "vorläufig." This has been regarded by some bacteriologists as justification for disregarding the names as being provisional and not validly published. An Opinion may well be required to clarify the meaning of the pertinent section of the Bacteriological Code. That portion of Rule 12 reads:

> "A name of a taxonomic group is validly published only if it has been definitely accepted by the author who published it. A name proposed provisionally (nomen provisorium) in anticipation of the eventual acceptation of the group, or of the circumscription, position, or rank given to a group, or mentioned only incidentally is not validly published."

A name "not validly published" is without standing in nomenclature. Apparently this provision was the reason for the name Chlamydozoon bombycis von Prowazek being discarded by Holmes (1948, p. 1226) and for the recognition of the name Borrelina bombycis Paillot.

There has been no clear definition of "provisional" as used in the code. Some suggestions may be made:

1. When an alternative name is given by an author, without acceptance, it is regarded as not validly published. Beijerinck (1903, p. 217) in his manuscript proposing the generic name Azotobacter mentions that Parachromatium might also have been a suitable name. The latter name is invalid.

2. A name given incidentally or provisionally and later definitely discarded by the author and replaced by another is invalid. In a footnote describing a new species in part, de Petschenko (1910) suggested a name (Müllerina paramecii) which in a subsequent article (1911) was definitely discarded and another (Drepanospira muelleri) given with a more adequate description. In Opinion No. 10 (1954) of the Judicial Commission the former name was regarded as "provisional" and discarded.

3. The Botanical Code, which has a statement substantially the same as that in the Bacteriological Code, adds an explanation of the meaning of "definitely accepted by the author." This provision:

> "does not apply to names or epithets published with a question mark or other indication of taxonomic doubt, yet published and accepted by the author."

The author von Prowazek apparently felt a name to be needed, he gave one which he thought appropriate, but hedged somewhat by defining his position "without for the time being setting forth more accurately its systematic position." Apparently his concern was not with the name applied as being provisional but with the systematic position of the organism.

There seems to be justification for the conclusion that von Prowazek gave and accepted the name with full recognition that its place in the scheme of classification was uncertain, and further that the name Chlamydozoon bombycis von Prowazek was validly published.

The generic names Borrellina and Borrelina

The generic name Borrellina was given by Paillot to a group of organisms (viruses) regarded as the etiologic agents of the polyhedral diseases of various lepidopterous insects. Three species were named and described, the first being Borrellina bombycis, the cause of silkworm jaundice. No note is taken by this author of earlier names such as Chlamydozoon bombycis von Prowazek.

The generic name (with spelling Borrelina) was accepted by Holmes (1948, p. 1225). This author designated Borrelina bombycis as the type species, and recognized five species, two of them new. He also named a new family Borrelinaceae to include the two genera Borrelina and Morator. Concerning the change in spelling of the generic name, he states:

> "The genus Borrelina Paillot was originally spelled Borrellina by error; from Borrel, name of French scientist."

Holmes explains his acceptance of the generic name Borrelina Paillot rather than Chlamydozoon von Prowazek in the statement:

> "(Note: Coccus-like bodies surrounded by nonstaining substances, associated with the induced disease, received the provisional name Chlamydomonas bombycis from Prowazek, Arch. f. Protistenkunde, 10, 1907, 363.)"

If the generic name Chlamydozoon von Prowazek was validly published, as suggested above, it has priority over Borrelina Paillot, and the type species is Chlamydozoon bombycis von Prowazek. However, this conclusion is based on the possibly fallacious assumption that there were no legitimate names applied to the viruses of insect polyhedral diseases before the publication of Chlamydozoon. The existence of earlier names has been noted by several authors, including Hughes (1953) and Zhdanov (1953). A critical review of the status of the generic name Borrelina is needed.

Some Questions to be Considered

The following questions are under consideration by the Judicial Commission to determine whether Opinions are needed, and if needed what their contents should be.

1. Were the generic name Chlamydozoon and the species name Chlamydozoon bombycis von Prowazek validly published, or did the author's use of the designations "provisorisch" and "vorläufig" invalidate their publication and make them "without standing in nomenclature?"

2. On the assumption of validity of publication of Chlamydozoon and of Chlamydozoon bombycis, what is the name of the type species?

3. Are the species included in Chlamydozoon in Ed. 6 of Bergey's Manual without a generic name?

4. Is the correction of the specific epithet trachomae to trachomatis under the code a correction of a "clearly unintentional orthographic error?"

5. Was the change of the spelling of Borrellina to Borrelina in conformity with the code rules governing correction of spelling?

6. What further clarification is needed of the meaning of the phrase "name proposed provisionally (nomen provisorium)" as used in Rule 12 of the Bacteriological Code?

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BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

International Committee on Bacteriological Nomenclature

Notices from the Secretaries

Membership of the Nomenclature Committee. Nominations for membership are made by the national society of the country concerned; no country is entitled to have more than five members. The next Congress will probably be held in the summer of 1957, so that where changes in the national representation are to be made, the national society should inform one of the Permanent Secretaries before July, 1956.

New member nominated. Dr. L.M. Black, of the Department of Botany, University of Illinois, Urbana, Illinois, has been nominated a member of the International Committee on Bacteriological Nomenclature, by the Council of the Society of American Bacteriologists, acting as the national society representing all microbiologists in the United States.

Dr. Black's nomination has been received by the secretaries, and will be put before the next meeting of the Committee. In the meantime, Dr. Black is regarded as a temporary member of the Committee.

Amendment of the Bacteriological Code. "Proposals for amendment of the Bacteriological Code shall be submitted to one of the Permanent Secretaries at least one year before the next International Congress" (Provision 4, C.2). Proposals may be made by members of the Nomenclature Committee or by individual microbiologists through a member representing their country.

Notice from the Editorial Board

This BULLETIN deals with all facets of the problems of taxonomy and nomenclature, and contributions are invited from microbiologists. Thus descriptions of technical methods for the study of micro-organisms, surveys of the characters of groups of organisms, and the development of classifications, descriptions of new genera, species or serotypes, proposals for the selection of neotype cultures, with appropriate descriptions, are all suitable subjects for papers in this BULLETIN. In addition, discussions on the nomenclatural problems are always welcome.

The BULLETIN was started so that workers in different countries could express their views on nomenclatural and taxonomic problems that are outside the scope of the ordinary technical and scientific journals, and the Editorial Board hopes that interest will be increased by the publication of papers of rather wider scope than those which appeared in the earlier volumes.

Contributors are asked to send manuscripts in the English language to Professor R.E. Buchanan and those in French or German to Professor T. Wikén, whose addresses will be found on the back cover of the BULLETIN. The number of reprints required should be specified when manuscripts are submitted.

ERRATA

In the April issue of the BULLETIN (Vol. 5, No. 2, p. 90, 1955), alpha, beta, and gamma were inadvertently omitted from paragraphs 4, 5, and 6, respectively. These should have read:

4. Mycoplasma spumans sp. nov. α strains from dogs ....

5. Mycoplasma canis comb. nov. β strains from dogs .....

6. Mycoplasma maculosum sp. nov. γ strains from dogs ...

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